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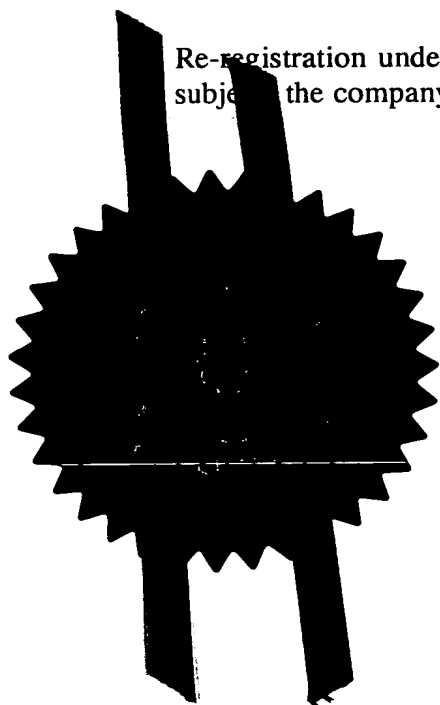
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Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

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4. Title of the invention
Vaccine

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Dr. Louise Sealy

[0117] 9260197

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VACCINE

This invention relates to an immunomodulator for use in a vaccine which is intended for use against a range of infectious agents. Further this invention relates to a vaccine composition comprising the immunomodulator, preferably in combination with antigen and a vaccination method using the vaccine composition.

Cholera toxin (Ctx) and its close relative E. coli heat-labile enterotoxin (Etx) are potent immunogens and mucosal adjuvants. E. coli verotoxin (Vtx) is another known bacterial toxin. The inherent toxicity of Ctx and Etx makes them unsuitable for human use. For example, although Ctx is the most commonly used mucosal adjuvant in experimental animals, it is unsuitable for use in humans because of its potent diarrhoea-inducing properties. Attempts have been made to separate toxicity from adjuvant activity, for example by using components of Ctx and Etx as replacements for the holotoxins themselves.

Ctx and Etx are heterohexameric proteins composed of a an enzymatically active A subunit and a pentameric B subunit. CtxB and EtxB are known to bind GM1-ganglioside (GM1), a glycosphingolipid found ubiquitously on the surface of mammalian cells.

In an attempt to circumvent the problem of toxicity for vaccine development, the adjuvant activity of the non-toxic B subunits has previously been investigated. However, many of the reports describe experiments in which a commercial preparation of CtxB or EtxB was used. These preparations are inevitably contaminated with a small but biologically significant amount of active toxin, so the adjuvant activity attributable to the B subunit is indistinguishable from the adjuvant activity of the whole toxin (Wu and Russell (1993) Infection and Immunity 61: 314-322, US-5182109). Subsequent studies using recombinant CtxB

(rCtxB) have suggested that CtxB is a poor mucosal adjuvant and only the addition of native holotoxin can provoke strong bystander responses (Tamura et al (1994) Vaccine 12: 419-426). Other studies have suggested
5 that rCtxB lacks the ADP-ribosylating and the CAMP-stimulating activities of the holotoxin and that, as adjuvant mechanism is linked to these abilities, CtxB would be unsuitable for use as an adjuvant (Vajdy and Lycke (1992) Immunology 75: 488-492, Lycke et al (1992)
10 Eur. J. Immunol. 22: 2277-2281, Douce et al (1997) Infection and Immunity 65: 2821-2828).

In another study, intranasal administration of ovalbumin using rCtxB as an adjuvant resulted in poor antibody responses. A non-toxic derivative of Ctx with
15 a mutation in the A subunit also generated weak responses to bystander antigens, whereas the presence of an active A subunit dramatically enhanced adjuvant activity, suggesting that an active A subunit is essential (Douce et al (1997) as above).

It has also been shown that rCtxB and rCtxB can be used to promote tolerance to heterologous antigens (Sun et al (1994) Proc. Natl. Acad. Sci. 91: 4610-4614, Sun et al (1996) Proc. Natl. Acad. Sci. 93: 7196-7201, Bergerot et al (1997) Proc. Natl. Acad. Sci. 94: 4610-
20 4614, Williams et al (1997) Proc. Natl. Acad. Sci. 94: 5290-5295), suggesting that these molecules would be unsuitable for use as adjuvants.

The basis of the present invention

30 In spite of the teaching in the art that CtxB and EtxB have poor adjuvanticity and can, in fact, act as tolerogens, the present inventors nevertheless investigated the use of rEtxB (thus containing no residual holotoxin or A subunit) in a vaccine for HSV
35 in a murine model and surprisingly found that it is able to stimulate protective immune responses to viral

challenge. Specifically, the present inventors found that:

- 5 i) agents such as ExtB and CtxB which bind GM-1 stimulate high levels of local (mucosal) antibody production (although immunization using rEtxB stimulated lower levels of overall serum antibody production than Ctx/CtxB combined);
- 10 ii) agents which bind the glycolipid Gb3 receptor, such as the B-subunit of E.coli verotoxin (VtxB) also induced high local antibody production.
- iii) agents such as EtxB, CtxB and VtxB also stimulated local T-cell proliferative responses;
- 15 iv) the distribution of antibodies produced was skewed towards non-complement fixing antibodies, especially sIgA;
- v) agents such as CtxB, EtxB and VtxB tend to shift the immune response from a Th1-associated response towards a Th2-associated response;
- 20 vi) when agents such as CtxB, ExtB and VtxB are used as immunomodulators some of the harmful effects of Th2-associated responses, such as the generation of IgE, are avoided;
- vii) rEtxB is a more efficient immunomodulator than rCtxB;
- 25 viii) agents such as EtxB, CtxB and VtxB are capable of altering the way in which an antigen presenting cell internalises and processes antigen, increasing antigen persistence; and
- 30 ix) if an agent such as EtxB, CtxB and VtxB is linked to an antigen, it is possible to alter the processing route of the antigen by altering the linkage to the immunomodulator.

These important discoveries are the basis of the various aspects of the present invention and enabled
35 the inventors to predict that pure EtxB, CtxB and VtxB, as well as other agents capable of binding to or

mimicking the effect of binding to GM1 or Gb3, will be useful as immunomodulators for use in vaccines in the prophylactic and therapeutic vaccination against HSV-1 infection, as well as other infections, the prevention or treatment of which would benefit from immunomodulation of the types listed above.

GM-1 and Gb3-associated signalling

Without wishing to be bound by theory, it is believed that GM-1 or Gb3 binding may trigger intracellular signalling directly or indirectly. The present inventors have also found evidence which suggests that EtxB interacts with at least one other protein which is involved in the GM-1 associated intracellular signalling event.

Definitions

An adjuvant is a substance which non-specifically enhances the immune response to an antigen, as distinct from a vaccine carrier, the purpose of which is to target the antigen to a desired site. The term "immunomodulator" is used herein to indicate an agent which acts, like an adjuvant, to stimulate certain immune responses, but which also directs the immune response in a particular direction.

The term "coadministration" is used to mean that the site and time of administration of the antigen and immunomodulator are such that the necessary immune response is stimulated. Thus, while the antigen and the immunomodulator may be administered at the same moment in time and at the same site, there may be advantages in administering the antigen at a different time and/or at a different site from the immunomodulator.

The term "antigenic determinant" as used herein refers to a site on an antigen which is recognised by

an antibody. Preferably it is a short peptide derived from or as part of a protein antigen, however the term is also intended to include glycopeptides and carbohydrate epitopes. The term also includes modified sequences of amino acids or carbohydrates.

The terms "CtxB", "CtxB" and "CtxB" as used herein include natural and recombinant forms of the molecule. The recombinant form is particularly preferred. They also include mutant molecules and other synthetic molecules (containing parts of CtxB, CtxB or CtxB) which retain the desirable immunological properties of CtxB, CtxB or CtxB.

Stimulation of immune responses

CtxB, CtxB, CtxB and other agents capable of binding to or mimicking the effects of binding to GM1 or Gb3, are capable of acting as immunomodulators and stimulating specific immune responses to antigenic challenge.

According to a first aspect of the present invention, there is provided the use of:

- (i) CtxB, CtxB or CtxB free from whole toxin;
- (ii) an agent other than CtxB or CtxB, having GM1-binding activity, or an agent other than CtxB having Gb3-binding activity; or
- (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

as an immunomodulator for a vaccine against infectious diseases.

According to a second aspect of the present invention, there is provided a vaccine composition for use against an infectious disease, comprising an antigenic determinant and an immunomodulator selected from:

- (i) CtxB, CtxB or CtxB free from whole toxin;

(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

5 (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

wherein said antigenic determinant is an antigenic determinant of said infectious disease.

10 The antigen and immunomodulator may be linked, for example covalently or genetically linked, to form a single effective agent, although separate administration, in which the antigen and immunomodulator are not so linked is preferred in some circumstances because it enables separate
15 administration of the different moieties.

According to a third aspect of the present invention, there is provided a kit for vaccination of a mammalian subject against an infectious disease, comprising:

20 a) one of the following agents:

(i) EtxB, CtxB or VtxB free from whole toxin;
(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

25 (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding; and

b) an antigenic determinant which is an antigenic determinant of the infectious disease, for
30 coadministration with the said vaccine immunomodulator.

The vaccine composition of the second aspect of the invention and the kit of the third aspect of the invention may be used in a prophylactic or therapeutic vaccination method, where a "prophylactic vaccine" is
35 administered to naive individuals to prevent disease development, and a "therapeutic vaccine" is

administered to individuals with an existing infection to reduce or minimise the infection or to abrogate the immunopathological consequences of the disease.

According to a fourth aspect of the present invention there is provided a method of preventing or treating a disease in a host, which method comprises the step of inoculating said host with a vaccine comprising at least one antigenic determinant and an immunomodulator, where the immunomodulator is:

- (i) EtxB, CtxB or VtxB free from whole toxin;
- (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding.

The vaccine may be administered by a number of different routes such as intranasal, oral, intra-vaginal, urethral or ocular administration. Intranasal immunisation is preferred.

The antigenic determinant and immunomodulator may be administered to the subject as a single dose or in multiple doses.

Stimulation of mucosal immune responses

EtxB, CtxB, VtxB and other agents capable of binding to or mimicking the effects of binding to GM1 or Gb3, are capable of specifically upregulating mucosal antibody production.

The vaccine immunomodulator of the first aspect of the invention, the vaccine composition of the second aspect of the invention and the kit of the third aspect of the invention are particularly effective against diseases where protection from infection or treatment is effected *in vivo* by a mucosal immune response. For example, against diseases in which, during infection,

the infectious agent binds to, colonises or gains access across the mucosa. Examples of such diseases include, diseases caused by viruses (HIV, HSV, EBV, CMV, influenza, measles, mumps, rotavirus etc),
5 diseases caused by bacteria (E. Coli, salmonella, shigella, chlamydia, N-gonorrhoea, T. pallidum, Streptococcus species including dental caries), and diseases caused by parasites.

10 In a preferred embodiment of the second aspect of the present invention there is provided a vaccine against HSV-1 infection comprising at least one HSV-1 antigenic determinant and an immunomodulator, where the immunomodulator is:

- (i) EtxB, CtxB or VtxB free from whole toxin;
- 15 (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3
20 binding.

Preferably the immunomodulator is EtxB.

In a preferred embodiment of the third aspect of the present invention there is provided a kit for vaccination of a mammalian subject against an HSV-1,
25 comprising:

- a) a vaccine immunomodulator which is:
 - (i) EtxB, CtxB or VtxB free from whole toxin;
 - (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB
30 having Gb3-binding activity; or
 - (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding; and
- b) at least one HSV-1 antigenic determinant,
35 for coadministration with the said vaccine immunomodulator.

According to a fifth aspect of the invention there is provided the use of:

- (i) EtxB, CtxB or VtxB free from whole toxin;
- (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding

to upregulate the production of antibodies at mucosal surfaces.

The antibodies produced in accordance with the fifth aspect of the invention are predominantly non-complement-fixing serum antibodies. Preferably, sIgA is produced in accordance with the fifth aspect of the invention.

In this fifth aspect of the present invention, the agent may be used in conjunction with one or more antigenic determinant(s).

Downregulating the pathological components of immune responses

The inventors also found that when pure EtxB was used as an immunomodulator in the described way, the harmful effects of Th2 associated responses, such as the generation of high levels of potentially pathological IgE, were avoided. The immune response triggered by the use of EtxB (or CtxB or VtxB) as an immunomodulator appears to favour the induction of Th2-associated cytokines. In other words EtxB (or VtxB or CtxB) induces a shift from a Th1- to a Th2-type response. This has enabled the inventors to predict that pure EtxB, CtxB or VtxB, as well as other agents capable of binding to or mimicking the effect of binding to GM1 or Gb3, will be capable of down regulating pathological components of the immune

response associated with both Th1 and Th2 activation.

According to a sixth aspect of the present invention, there is provided the use of:

- (i) EtxB, CtxB or VtxB free from whole toxin;
- 5 (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3
- 10 binding;

to downregulate the pathological components of Th2-associated immune responses. The pathological components of Th1-associated immune responses may also be downregulated.

15 It is known that EtxB and CtxB bind to GM1 and induce differential effects on lymphocyte populations, including a specific depletion of CD8+ T cells and an associated activation of B cells (WO 97/02045). Hence, EtxB and CtxB are thought to alter the balance of the

20 immune response such that inflammatory Th1 associated reactions are down-regulated while Th2 associated responses are upregulated. Th1 responses include the secretion of γ IFN by activated T-cells leading to macrophage activation and delayed type hypersensitivity reactions. Such responses may be an important cause of

25 pathology during infections with a number of pathogens. Th2 responses include the activation of T-cells to produce cytokines such as IL-4, IL-5, IL-10, and are known to promote the secretion of high levels of

30 antibody, especially IgA.

It has now surprisingly been found that when EtxB is used as an immunomodulator in the described way, the harmful effects of Th2 associated responses, such as the generation of high levels of potentially

35 pathological IgE, are avoided. Therefore, EtxB and CtxB are capable of down regulating pathological

components of the immune response associated both with Th1 and Th2 activation. Such responses are modulated in favour of the production of high levels of non-complement fixing serum antibodies and secretory IgA production at the mucosal surfaces.

The use of an agent in accordance with the sixth aspect of the invention is particularly useful to treat diseases in which immunopathological mechanisms are involved. Examples of such diseases are HSV-1, HSV-2, TB, HIV, leprosy and leishmania.

The first and sixth aspects of the invention can be combined. In other words, agents such as EtxB can be used simultaneously as an immunomodulator and a therapeutic agent. For example in diseases where immunopathological mechanisms are involved, the use of a vaccine incorporating agents such as EtxB or CtxB may act not only to limit infection, but also to abrogate the disease. The immunomodulating agent is thus acting both prophylactically and therapeutically. Examples of infections where vaccination in this way is therefore likely to be of particular value include those caused by the herpes virus family, measles, gastrointestinal and respiratory tract pathogens.

Immunomodulation of the antigen processing pathway

a) prolonging presentation

The present inventors have also found that when EtxB (or CtxB or VtxB) is used as an immunomodulator in a vaccine, the antigen internalisation and processing pathway is altered. The presence of the B subunit causes prolonged presentation, possibly due the antigen presenting cell storing internalised antigen for unusually long periods in the form of "antigen deposits". This feature of B-subunit associated antigen presentation means that vaccines incorporating an agent in accordance with the present invention will

have increased antigen persistence and sustained immunological memory.

According to a seventh aspect of the present invention, there is provided the use of:

- 5 (i) EtxB, CtxB or VtxB free from whole toxin;
 (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
10 (iii) an agent having an effect on intracellular signalling events mediated by GM1-binding or Gb3 binding;

as an immunomodulator in a vaccine, to prolong antigen presentation and give sustained immunological memory in a mammalian subject.

- 15 According to an eighth aspect of the present invention, there is provided a vaccine composition for use against an infectious disease, comprising an antigenic determinant and a immunomodulator selected from:

- 20 (i) EtxB, CtxB or VtxB free from whole toxin;
 (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
 (iii) an agent having an effect on intracellular
25 signalling events mediated by GM1-binding or Gb3 binding;

wherein said antigenic determinant is an antigenic determinant of said infectious disease and wherein the immunomodulator prolongs presentation of the antigenic
30 determinant and gives sustained immunological memory.

b) intracellular targeting of the antigen to a MHC-I or MHC-II associated pathway

35 As aforementioned, the antigen and immunomodulator in a therapeutic or prophylactic vaccine may be linked, for example covalently or genetically linked, to form a

single effective agent. The present inventors have found that it is possible to direct the antigen to different compartment of the cell and hence to different antigen presentation pathways by altering the linkage of the antigen to the immunomodulator.

By linking the antigen or antigenic determinant to the immunomodulator in a certain way, it is possible to facilitate translocation of the antigen across the endosomal membrane into the cytosol, and hence enhance loading of antigenic peptides on to MHC class I molecules. The use of an antigen-immunomodulator conjugate can therefore both down regulate the immunopathological components of Th1-associated immune responses (including δ IFN-induced macrophage activation and DTH responses) and activate cytotoxic T cells (CTL). Induction of CTL is beneficial for the prevention and treatment of many diseases especially those caused by viruses, intracellular bacteria and parasites.

The linkage of the antigen-immunomodulator conjugate can also be chosen so that the antigen is delivered into the nucleus.

According to a ninth aspect of the present invention there is provided a conjugate comprising an antigen or antigenic determinant and an immunomodulator selected from:

(i) EtxB, CtxB or VtxB free from whole toxin;
(ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or

(iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding.

According to a tenth aspect of the present invention there is provided a vaccine composition for use against an infectious disease, comprising a conjugate of an antigen or antigenic determinant and an

immunomodulator selected from:

- (i) EtxB, CtxB or VtxB free from whole toxin;
- (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- (iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or G3b binding; wherein said antigen or antigenic determinant is an antigen or antigenic determinant of said infectious disease.

The antigen or antigenic determinant may be linked to the immunomodulator by a variety of methods including genetic linkage or chemical conjugation. In a first preferred embodiment the conjugate is a fusion protein made by genetic linkage of the antigen or antigenic determinant to the immunomodulator. Preferably the antigen or antigenic determinant is genetically linked to the C-terminus of the immunomodulator. In a second preferred embodiment the antigen or antigenic determinant is chemically conjugated to the immunomodulator. Preferably the antigen or antigenic determinant is conjugated to the immunomodulator using heterobifunctional cross-linking reagents. More preferably the cross-linking agent is N-(δ -maleimido-butyroxy)-succinimide ester (GMBS) or N-succinimidyl-(3-pyridyl-dithio)-propionate (SPDP).

According to an eleventh aspect of the present invention there is provided the use of:

- (i) EtxB, CtxB or VtxB free from whole toxin;
- (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- (iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding; in a conjugate with antigen or antigenic determinant to target the delivery of said antigen or

antigenic determinant to the cytosol or nucleus of an antigen presenting cell.

According to a twelfth aspect of the present invention there is provided the use of:

- 5 (i) EtxB, CtxB or VtxB free from whole toxin;
- (ii) an agent other than EtxB or CtxB, having GM1-binding activity, or an agent other than VtxB having Gb3-binding activity; or
- 10 (iii) an agent which has an effect on vesicular internalisation mediated by GM1-binding or Gb3 binding; in a conjugate with antigen or antigenic determinant to upregulate the presentation of said antigenic determinant, or an antigenic determinant derived from said antigen, by MHC class I molecules.

15

It has previously been thought that EtxB and CtxB have similar properties. However, the present inventors have found that rEtxB is a more potent and efficient immunomodulator than rCtxB. Hence the preferred immunomodulator is EtxB, or agents which

20 mimic the effects of EtxB.

Agents other than EtxB, and CtxB which retain GM1 binding activity and agents other than VtxB which

25 retain Gb3 binding activity include antibodies which bind GM1 and Gb3. Humanised monoclonal antibodies are especially preferred.

In all aspects of the invention, the agent having GM1- or Gb3-binding activity may also be capable of

30 cross-linking GM1 or Gb3 receptors. EtxB is one such agent which is capable of cross-linking GM1 receptors by virtue of its pentameric form.

In all aspects of the present invention, more than one agent may be used in combination.

35

The invention will now be illustrated by reference

to the accompanying drawings and the following examples.

The examples refer to the figures in which:

5 Figure 1: shows the level of Ig or IgA in MS or IgA in EW compared with control mice following immunisation with HSV-1 or Mock Gp preparations with different amounts of rEtxB.

10 Figure 2: shows T cell proliferation of MLN or CLN lymphocytes in mice immunised intranasally with HSV-1/rEtxB.

Figure 3: shows T cell proliferation of cells from MLN and CLN of mice immunised intranasally with HSV-1 Gp in the presence of 1-20 μ g EtxB.

15 Figure 4: compares virus shedding, clinical disease and latency in mice immunised with HSV-1/rEtxB and control mice.

20 Figure 5: shows the Ig isotype distribution in MS following infection with HSV-1 or immunisation with HSV-1 Gp in the presence of EtxB or CtxB as immunomodulator.

Figure 6: shows the distribution of Ig subclasses following intranasal administration of HSV-1 Gp with either rEtxB or rCtxB as immunomodulator.

25 Figure 7: shows the immunogenic effect of different amounts of rEtxB or rCtxB on the level of HSV-1 specific IgA in eye washings following administration with HSV-1 glycoproteins.

30 Figure 8: shows the level of anti-HSV-1 serum Ig in mice following administration of HSV-1 glycoproteins three times at 10 day intervals with variable amounts of rEtxB or rCtxB as adjuvant.

Example 1: rEtxB can be used in conjunction with HSV-1 Gp for immunisation.

35 Mice were immunised intranasally with HSV-1 Gp and 10 μ g rEtxB (Group A), HSV-1 Gp and 20 μ g rEtxB (Group B)

or Mock Gp and 20µg rEtxB (Group C). The production of total Ig and IgA in MS and EW was stimulated by HSV-1 GP/rEtxB (Figure 1). Also, MLN and CLN T-lymphocytes from immunised mice were shown to proliferate when
5 cultured in vitro with HSV-1, but not when cultured in vitro with mock HSV-1 Gp or without antigen (Figure 2). The proliferation in response to HSV-1 Gp of T lymphocytes from MLN and CLN of mice immunised with HSV-1 Gp and varying amounts of EtxB is shown in Figure
10 3. Finally, Group A and B mice (as described above) were shown to have a decrease in virus shedding, clinical disease and latency than group C mice (Figure 4).

15 Example 2: rCtxB and rEtxB direct the immune response in a particular direction. The Ig isotype distribution and distribution of Ig subclasses following immunisation using EtxB or CtxB as an immunomodulator is shown in Figures 5 and 6.

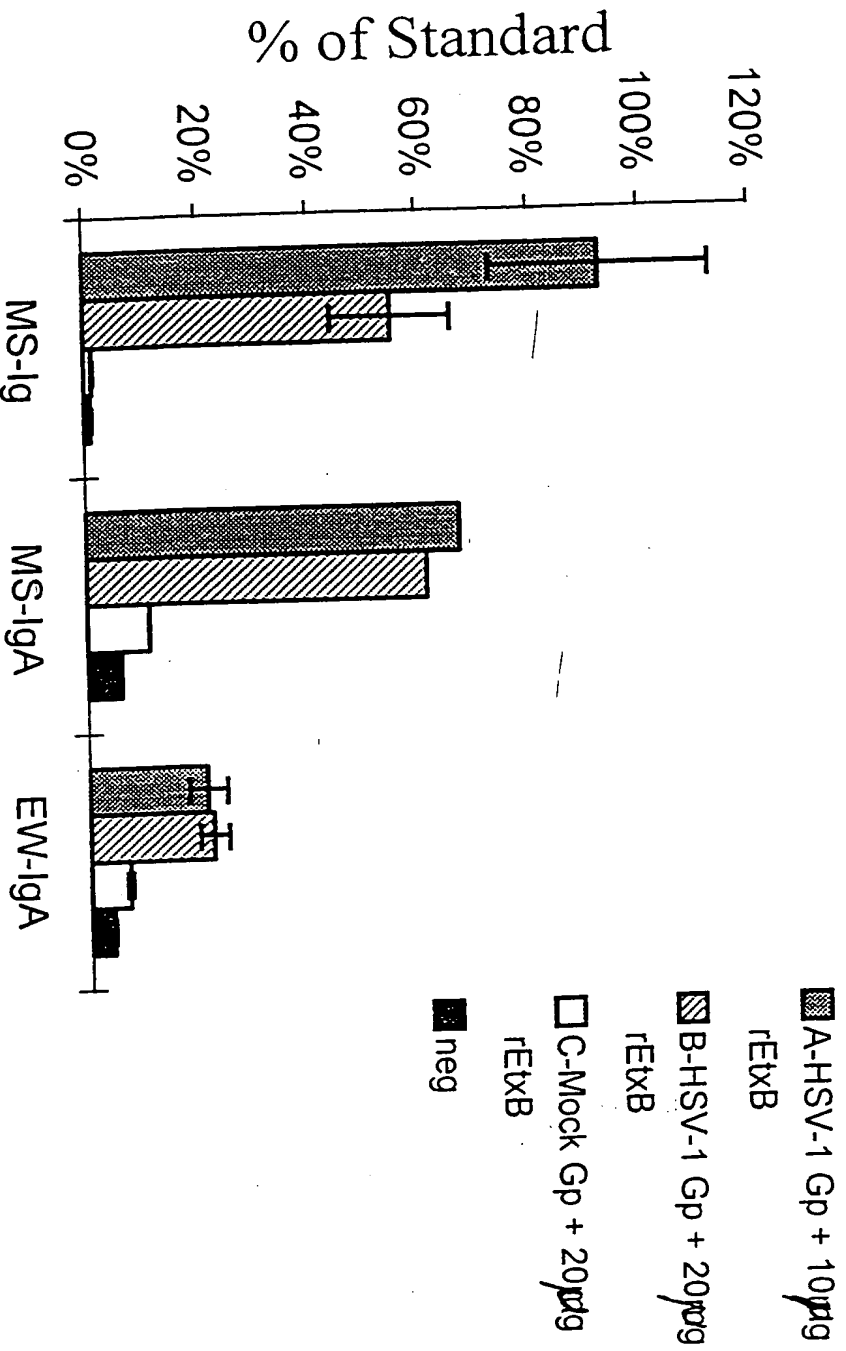
20 Example 3: rEtxB is a more efficient immunomodulator than rCtxB.

25 The levels of HSV-specific IgA (Figure 7) and total anti HSV-1 serum Ig (Figure 8) are greater following stimulation with rEtxB/HSV-1 Gp than rCtxB/HSV-1 Gp.

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Figure 1

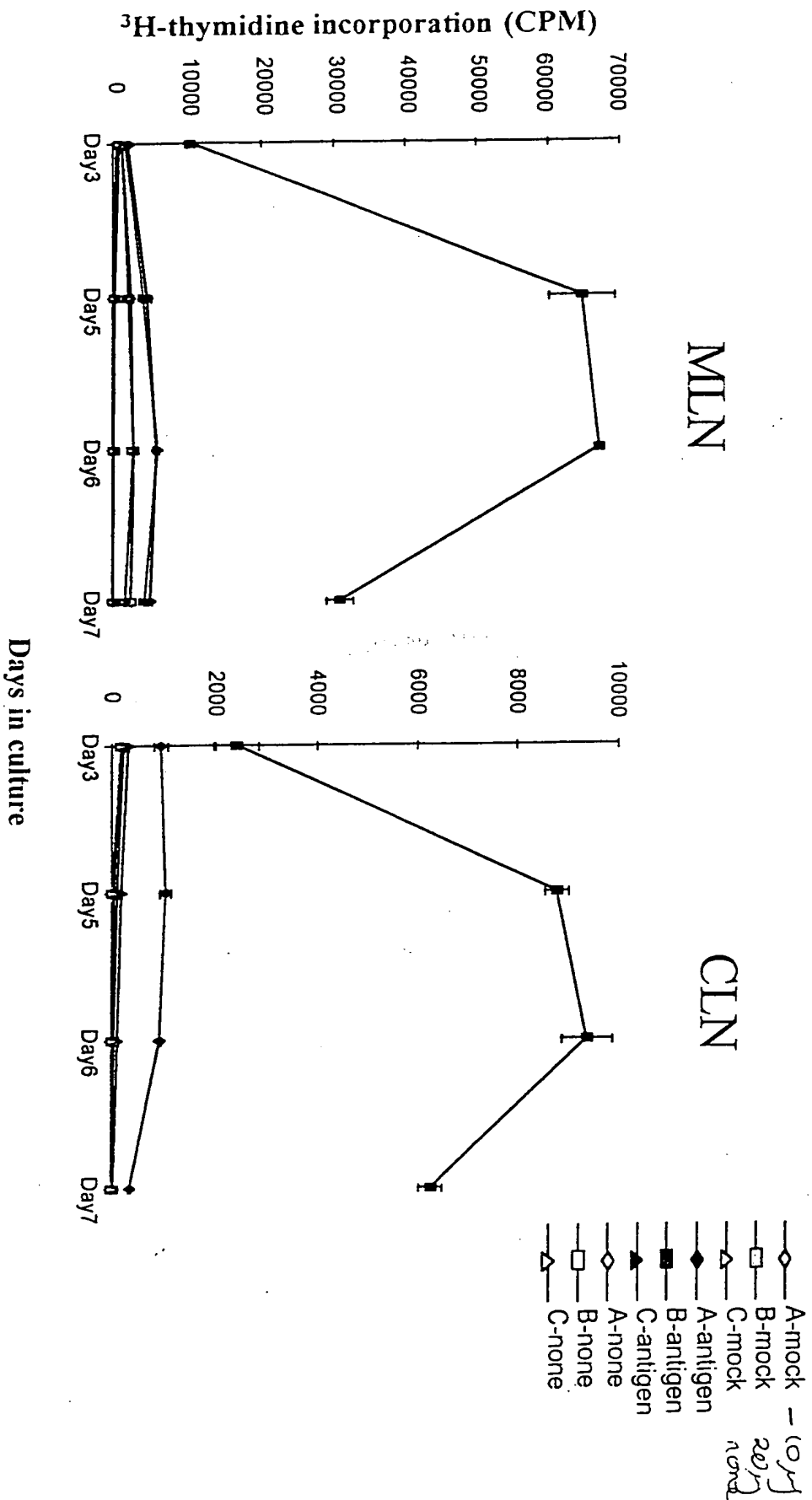
Level of Ig or IgA in MS or IgA in EW compared with control mice following immunisation with HSV-1 or mock Gp preparations with different amounts of rEtXB



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Figure 2

T cell proliferation of MLN or CLN lymphocytes from mice given HSV-1 GP with 10 μ g (A), 20 μ g (B) rEtXB or mock GP with 20 μ g rEtXB (C) by the i.n. route cultured *in vitro* with different antigens



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Cell proliferation of cells from MLN and CLN of mice immunised i.n. with HSV-1 Gp in the presence of 1-20 µg EtxB as adjuvant

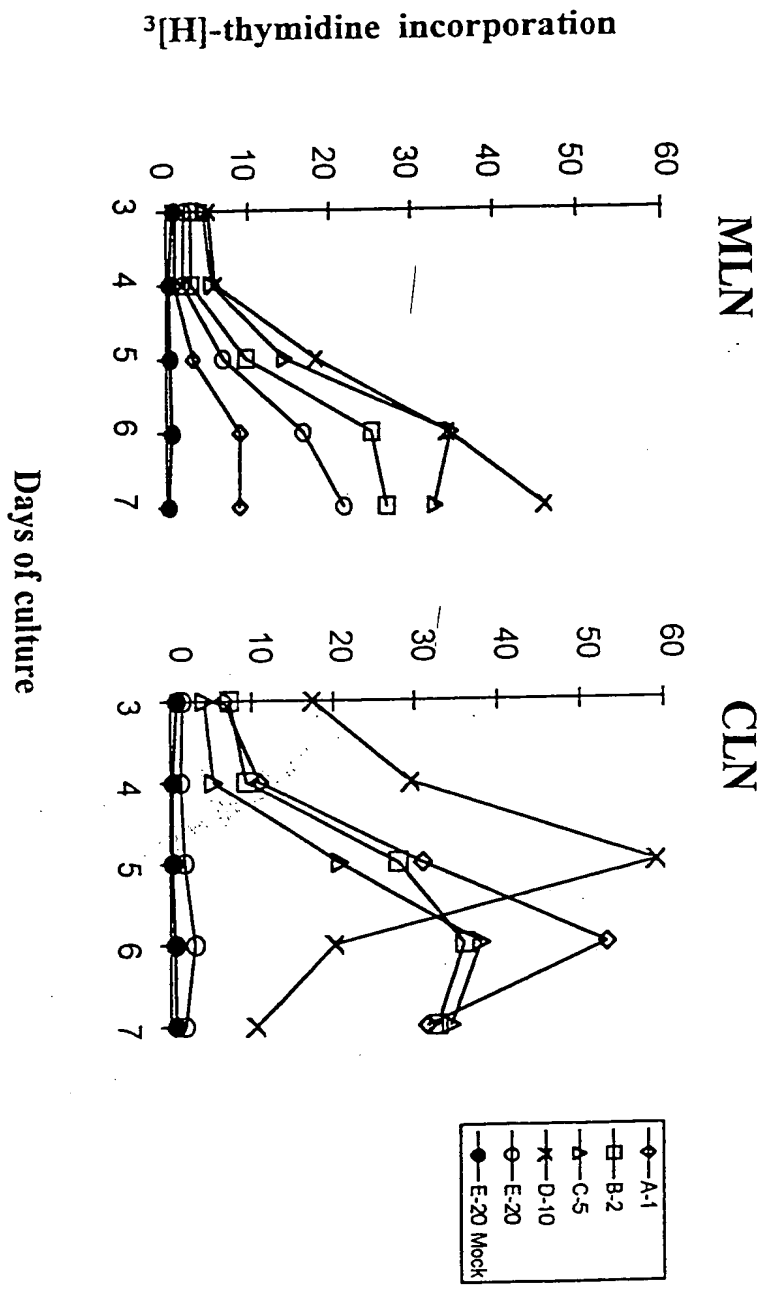


Figure 3

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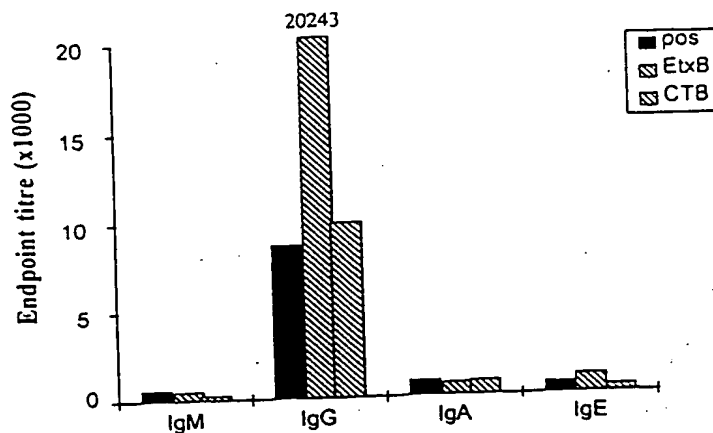
Figure 4

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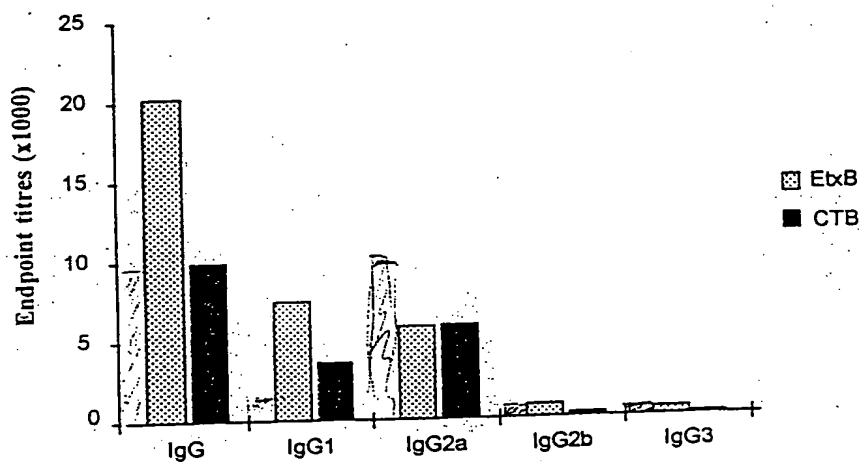
Ig Isotype distribution in MS from mice following infection (pos) or immunisation with HSV-1 Gp in the presence of EtxB or CTB as adjuvant

Figure 5



Distribution of subclasses following administration of HSV-1 Gp i.n. with either rEtxB or rCTB as adjuvant

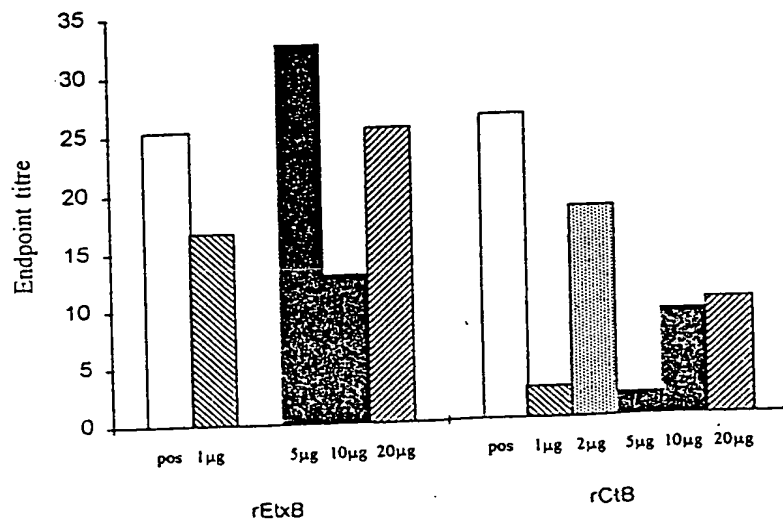
Figure 6



IgG and subclasses

Adjuvant effect of different amounts of rEtxB or rCTB on the level of HSV-1 specific IgA in eye washings following administration with HSV-1 glycoproteins

Figure 7



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Anti-HSV-1 serum Ig in mice following administration of HSV-1 glycoproteins three times at 10 day intervals with variable amounts of rEtxB or rCTB as adjuvant

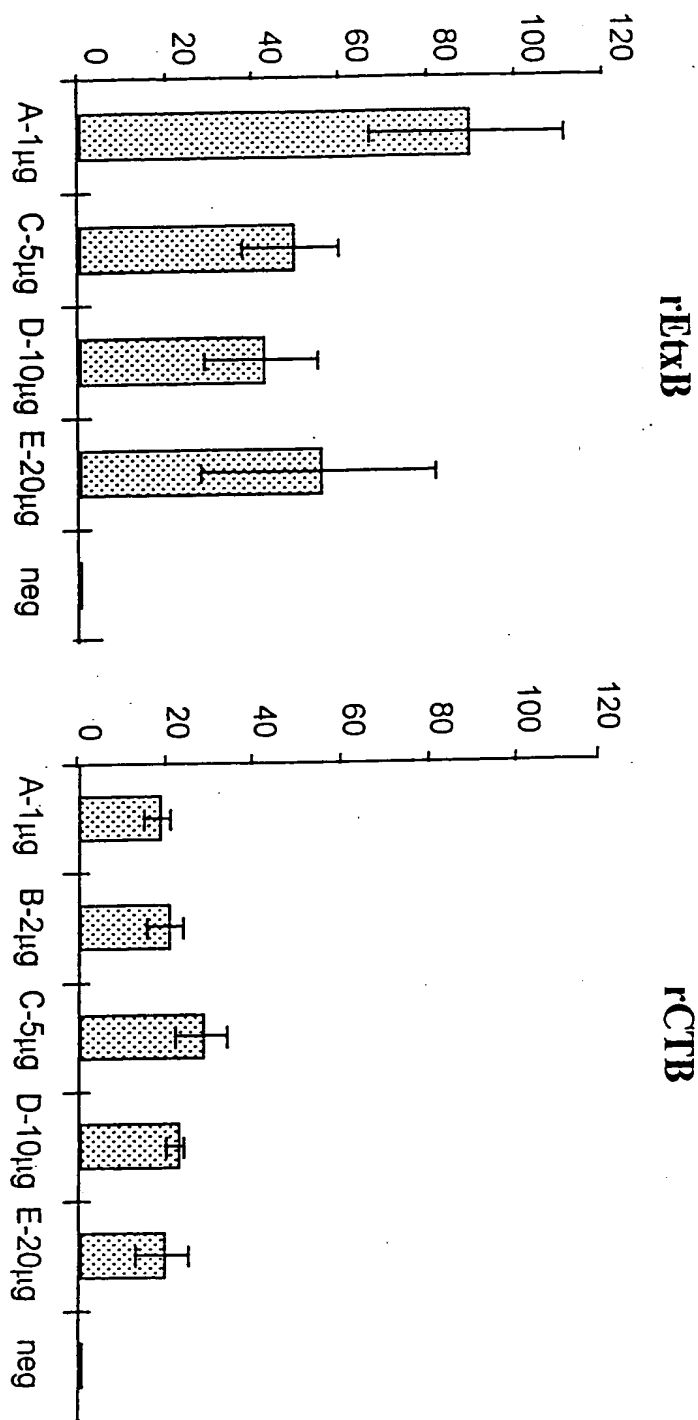


Figure 8

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